Scanning Electron Microscopy of Planktonic Larval Marine Gastropod Shells

BY

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(9 Plates)

Dedicated to the Memory of Dr. GUNNAR THORSON, who died 25 January 1971

INTRODUCTION

The Scanning (or Stereoscan) Electron Microscope (hereafter SEM) yields photographs of solid surfaces at magnifications below ×100 to above ×30,000 and with high resolution (KIMOTO & RUSS, 1969); the depth of field is up to 500 times that attainable with regular light photography or microscopy. For studying and illustrating surface features of small molluscan shells, the SEM is an excellent tool and yields information unobtainable in any other way (SOLEM, 1970). The first malacological application of the SEM was by RUNHAM & THORNTON (1967) in a study of the mechanical wear of gastropod radulae. Subsequently, the SEM has been used with greatly increasing frequency in studies of radulae as well as of gastropod, bivalve and cephalopod shell structure and external sculpture (e.g., WISE, 1970). SOLEM plans to review SEM studies of radular structure and function. One of the first molluscan soft tissues to be studied were sea hare (*Aplysia*) nerves (LEWIS *et al.*, 1969); these required dehydration techniques. TURNER & JOHNSON (1969) have used a SEM in studies of larval and young postlarval bivalve shells (*Lyrodus* shipworms), but so far there has been no reported use of the SEM in studies of larval gastropods.

My use of the SEM has been primarily in studies of planktonic larval gastropod shells and opercula. My primary objective has been to observe and illustrate the morphology of certain larval shells and opercula, especially their microsculpture, in order to aid specific identifications and to find new systematic characters. A secondary objective has been to try and find larval shell features recording early life history stages. The present paper records in detail the methods I have found most efficient and successful, and gives a few examples of the kinds of information that can be obtained. The examples have deliberately been chosen from families other than the Architectonicidae, the group to which most of my attention has been devoted. The latter work is still in progress and will be published elsewhere. The larval shells selected here have all previously been studied with light microscopy. Two of the species (*Smaragdia viridis* and *Pedicularia* sp.) were studied by SCHELTEMA (in press) in a study of transatlantic transport of larval gastropods by ocean currents.

ACKNOWLEDGMENTS

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METHODS

Veligers freshly collected from plankton generally have better shells for scanning electron microscopy than do veligers that have been preserved in ethyl alcohol or formalin. Both preservatives are acidic and etch or decalcify calcium carbonate shells. Shell surfaces are sometimes damaged in either preservative even when buffered, and small particles tend to adhere to some periostracums. Specimens are best handled and cleaned manually with fine paintbrushes. For cleaning, fine needles (Minuten-Nadeln) and jets of distilled water in successive changes of water are also necessary. My experiments with ultrasonic cleaning have been unsuccessful because the larval shells tried thus far have been too fragile and have quickly shattered. Pressurized anti-static gases are useful for cleaning containers and tools, and for drying and removing dust from specimens (beware the potency of the gas jet).

To provide a smooth background surface and to facilitate removal of specimens intact from the metal stub after study, I use specially cut glass cover slips (8 mm in diameter and 0.2 mm thick). A glass is glued to the 10 mm diameter metal stub with rubber cement. To ensure conductivity to the stub, the glass is ringed with colloidal silver suspended in toluene. Wider stubs can be used, but I have found them inconvenient.

I have experimented with a variety of mounting media, including double-stick transparent tape, colloidal silver, rubber cement, and *Duco* glue, and have found that polyvinyl acetate glue[2] is better than the others for small shells. It is viscous and white, develops a coat as it contracts and dries, is water soluble even after it dries, and when dry, is smooth and takes gold-coating well. Specimens are best mounted on small individual mounds of the glue that have begun to dry. Each shell should be gently pushed into the surface coat and yet not have glue submerging the edges. If a specimen sinks too deeply into the glue it can be retrieved by thorough re-washing in several changes of distilled water. Too narrow a pedestal of glue is to be avoided because the glue cannot be properly coated with gold and this results in "charging up" (white flecks across the picture). After studying one side of a specimen it is possible (with water soluble glue) to remount it the other way up. The layer of gold has to be broken to allow water to penetrate and soften the glue, and the shell has to be thoroughly recleaned before remounting. Small opercula and tiny larval shells need no mounting medium if upon drying they adhere firmly to the glass surface. Once specimens have been gold coated I have found that they can be restudied months later with no apparent changes if the stub is kept in a covered container.

The SEM model used was a Jeolco JSM-2, which allows stub rotation of 180° in a horizontal plane, movement along x and y axes, and tilting 2° from vertical to the left and 45° from vertical to the right. (The Cambridge scanning electron microscopes have the advantages of 360° stub rotation and a 90° tilt from vertical.) After horizontal stub rotation, controls move the stub obliquely (or with complete rotation in either direction the axes are reversed). The secondary electron beam detector lies to the right of the stub, and as a result of this the apparent light source on the screen is on the right. Thus, specimens can be tilted more than 2° from vertical only towards the apparent light source. Best results are generally achieved when surfaces are tilted towards the beam detector and when there are no objects between the subject and detector.

To aid comparisons, consistent orientation of larval gastropod shells is important. Mounting should therefore be carefully planned with the above considerations in mind. As many as ten or twenty small shells can be mounted on one stub, but they need to be placed well apart. It is best to plan on tilting the stub slightly and on not needing to rotate it much. Ideally, all the specimens on one stub should need the same orientation. This requires deciding in advance on the desired "lighting" and orientation of each shell. I have found it helpful after mounting to make a camera lucida sketch map of the relative positions and orientation of each specimen on the whole stub and the planned location of the right hand side. The map greatly facilitates specimen location, notes about each specimen can be made directly on the map, and each photograph can be related by number with a given shell.

Gastropod shells are insufficiently conductive to be studied in the SEM without metal coating. My experiments with uncoated shells led to charging up, electron beam damage, and strongly contrasting image patterns unrelated to surface morphology (but possibly related with variations in chemical composition). Thereafter, all specimens were coated with a layer of gold approximately 200 Å thick applied in a vacuum evaporator while the stub was slowly rotated around an axis about 45° from the vaporized gold source. I have rarely had charging problems with properly gold-coated specimens; problems can sometimes be circumvented by reducing the beam current to the condenser lens from 10^{-12} amps to 10^{-13} or 10^{-14} amps (this requires refocusing). I have

[2] Trade names include *Carter's Nu-Glu, Elmer's Glue-All,* and *U. S. Plywood White Glue.*

not tried other metals (palladium, platinum, and aluminum). I once tried carbon coating preceding the gold coating, a procedure which is supposed to help make the gold adhere better, but this yielded poor results—probably because the layer of carbon was too thick. The 200 Å thick layer of gold is thinner than the maximum attainable resolution with a commercially available SEM (about 250 Å) and therefore has no observable effect on surface sculpture. A method of removing the layer of gold from microfossils, using sodium cyanide, has been described (Hansen, 1968). I have not tried this but it should work with mollusk shells.

Accurate placement of the whole stub in its holder is important, both with regard to positioning of the right hand side and height of the top of the stub. If the highest specimens project more than about 1 mm above the top of the holder, they can too easily hit the top of the entrance to the sample chamber. Even with the 10 mm diameter stub at the lowest usual magnification (×100) it is easy to become confused as to which part of the stub is being scanned or which specimen is under observation. The sketch map mentioned earlier plus the television accessory are helpful for locating individual specimens. Even with series of identical specimens that have been preserved, cleaned and mounted in the same way, there usually is as yet unexplained variation in the degree of image contrast. Low-contrast specimens give the best results, and lack the contrasting light and dark bands parallel to smooth edges that are unavoidable with high-contrast specimens.

Standardized magnifications simplify size comparisons of identical structures on series of specimens. On the other hand, it is desirable to enlarge the particular area being observed so that it nearly fills the screen because the photographic negatives do not enlarge well. Some magnifications read from the dials have been accurate to within several percent, but the great depth of field is a source of error. I most frequently used magnifications of ×100, ×300, ×600, ×1000, and ×5000. Magnifications below ×100 can be achieved by lowering the stage. Focusing is done at higher magnifications than that at which photographs are taken. Views that include great topographic heights and depths need to be focused near the average height, and apical views and the like therefore should not be focused at too high magnifications. Clockwise rotation of the focus controls moves the focus upwards. Above magnifications of ×1000, moving the stub with the manual controls becomes impractical on account of their coarseness and backlash. Instead, the image on the screen should be moved electronically (the vertical and horizontal fine shift controls need to be centered before beginning this). With the Jeolco JSM-2, I have consistently used the recommended accelerating voltage of 25 kilovolts. For viewing, I have found the optimum scan speed to be one second.

I have consistently used Polaroid positive-negative film (Type 55). Exposure and contrast have been estimated visually by the technicians, using the brightness and level contrast controls (sometimes after changing the beam current or detector power supply). For numbering the prints and negatives, I assign a letter denoting the particular work session with the SEM, and this is followed by a number for each photograph. The easily scratched Polaroid negatives are best kept in individual envelopes.

Visual traverses are useful for detecting morphologic features at high magnifications. These are done with one's right hand on the medium focus control and one's left hand slowly turning the x or y axis control.

First whorl diameters are based on a straight line superimposed on or closely paralleling the nearly straight beginning of the suture; the line is extended in each direction to where it intersects the nearest suture. The *embryonic shell* is that part of the larval shell grown before the larva hatches from an egg mass or egg capsule. A *protoconch* is a larval shell attached to a postlarval shell (*teleoconch*). Measurements are given either in *millimeters* (mm) or *microns* (μ).

MATERIALS

The larval shells studied here were all obtained from oceanic plankton collected southeast of Bermuda during April, 1970. An area at 32° 10′ N and 64° 30′ W, 26 km (14 nautical miles) southeast of Castle Roads, was visited on April 2 and April 14 in the research vessel *Panulirus II* (stations 321 and 322), and each time two plankton tows were made from the surface down to depths of about 80 to 130 m during midday and mid-afternoon. The bottom depth in the area is 2,900 to 3,100 m. The plankton net used was 1 m wide, 3.5 m long, and had #2 mesh (aperture diameter 0.37 mm) towards the opening and #8 mesh (aperture diameter 0.20 mm) at the cod end. Each tow lasted 20 to 25 minutes with the net towed at 4 km/hour (2 knots); the net was near the maximum depth for 10 to 20 minutes. So as to keep most of the plankton alive, it was kept cooler than the sea surface temperature (19° C) by surrounding the containers with melting ice, and upon return to the laboratory, the larval gastropods were sorted out as quickly as possible.

The comparisons of larval shells with protoconchs are based primarily on the following lots of postlarval specimens:

Smaragdia viridis, dredged 7 to 9 m, Castle Roads, Bermuda, June 23, 1951, Richard W. Foster (ANSP 267608). Specimen from same lot illustrated by SCHELTEMA (in press, plt. 3, fig. q).

Litiopa melanostoma (none illustrated here), on floating *Sargassum, Turbinaria,* etc., N of Crocker sand bore (16°37′05″ N; 88°06′20″ W), British Honduras, August 17, 1961, Robert Robertson (ANSP 282720).

Alaba incerta, on filamentous algae, 4.5 m, Grand Anse Beach, SW Grenada, Lesser Antilles, March 10, 1966, Virginia Orr Maes (ANSP 313755).

Janthina janthina, stranded on rocky shore NE of Hungry Bay, Paget Parish, Bermuda, March 31 and April 26, 1970, Robert Robertson (ANSP 320980).

Janthina pallida, stranded on rocky shore NE of Whalebone Bay, St. George's Island, Bermuda, April 24, 1970, Robert Robertson (ANSP 320981).

Pedicularia decussata (not studied with SEM), dredged 800 m, U. S. Fish Commission Sta. 2415, off Georgia, U.S.A. (USNM 108408). Same specimen illustrated by SCHELTEMA (in press, plt. 3, fig. c).

RESULTS

ARCHAEOGASTROPODA

NERITIDAE

Smaragdia viridis (Linnaeus, 1758)

(Plate 1)

A frequently encountered veliger in tropical and subtropical Atlantic Ocean plankton that is distinctive by its bright green digestive gland and its H-shaped velum has, at my suggestion, been identified by SCHELTEMA (in press, fig. 8 [map], plt. 3, figs. n–q) as this species, which is the only one in the family with an amphi-Atlantic distribution.[3] Optical and SEM comparisons show that the larval shell matches protoconchs of this species. The postlarval shells from the two sides of the Atlantic differ slightly in coloration, and the two populations are therefore supposed to be subspecifically distinct (RÉCLUZ, 1852: 283–284; RUSSELL, 1941: 397–398). SCHELTEMA shows, however, that transatlantic larval transport probably occurs in the westward-flowing South Equatorial Current. The green adults live on the leaves of marine monocotyledons.

In apertural view, the larval shell (Plate 1, Figure *1*) is oval in outline, smooth, and with a neritid-like aperture, but without columellar teeth or the wide parietal callus. In apical view, faint axial growth lines and plicae can be seen on the protoconch (Plate 1, Figure *2*) and at higher magnifications divaricating incised spiral lines 1 to 4 microns apart show near the apex (Plate 1, Figure *3*). A peculiar feature seen on all three specimens studied is a deeply immersed embryonic shell. The beginning of the suture is also immersed so that the visible portion of the embryonic shell is wholly surrounded by the suture and is an elliptic or polygonal area 53 to 82 microns long by 45 to 58 microns wide. The faint axial plicae and divaricating spiral lines of the embryonic shell are approximately at right angles to those on the first emergent part of the postembryonic shell. There therefore must be a major, gradual or abrupt change between the axes of coiling of the embryonic and postembryonic whorls. SCHELTEMA's figures (in press, plate 3, figures n and p) show this feature but this has not otherwise been noted in larval neritids.

On the operculum, SCHELTEMA mentioned but did not illustrate a "minute reinforcing bar." The SEM shows this to be a projection near the axis, with three supporting buttresses, one extending from the columellar margin; the apex projects laterally beyond the columellar margin (Plate 1, Figure *4*). The larval operculum is thus already typically neritid in having a projection that pivots beneath the lower part of the columella. Axial growth lines are present only on the exterior of the operculum, the interior being smoothly callused. On full-grown opercula, the projection widens and bears two apices, the basal one being knobby and upright and the other being the termination of a ridge.

[3] The Mediterranean veliger described and illustrated by THIRIOT-QUIÉVREUX (1970: 345 - 346; plt. 1, fig. 14; text fig. 1D) and unidentified by her ("Sp. A") appears to be *Smaragdia viridis.*

Explanation of Plate 1

Larval *Smaragdia viridis* (Linnaeus)

Figure *1*: Apertural view (× 150). The four circular pits are not normally present and are of unknown cause on this shell. ANSP 320982.

Figure *2*: Apical view of protoconch and part of surrounding teleoconch (× 150). ANSP 267608.

Figure *3*: The immersed and tilted embryonic shell surrounded by the suture and part of the postembryonic shell on another protoconch in apical view (× 600). ANSP 267608.

Figure *4*: Internal view of the paucispiral, calcareous operculum of the larva (× 220). ANSP 320982.

Figure 1

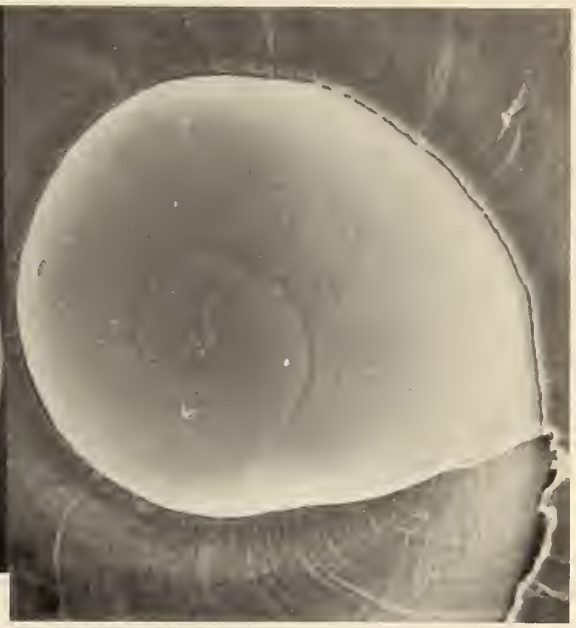

Figure 2

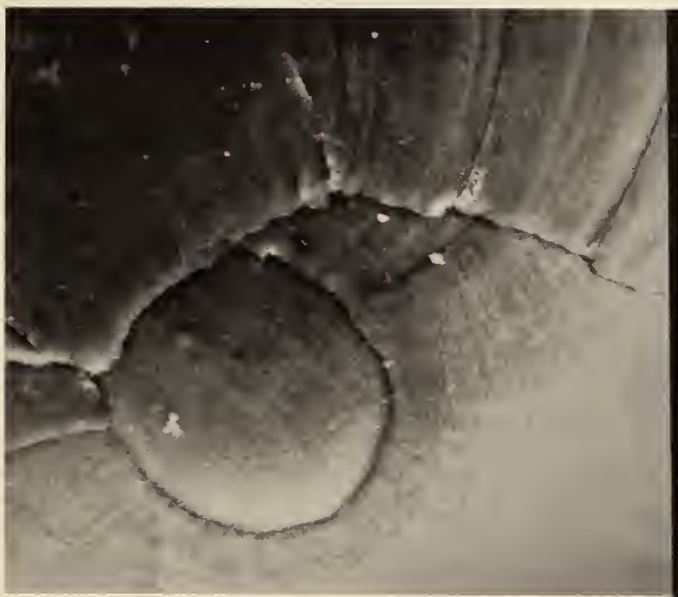

Figure 3

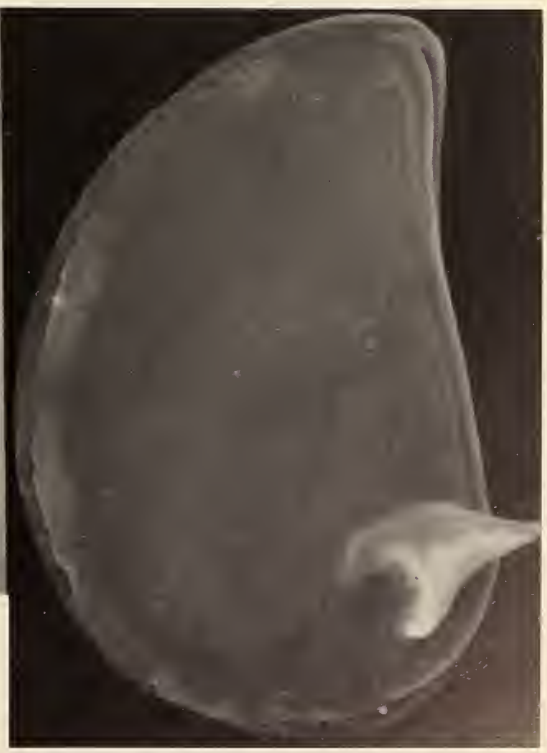

Figure 4

MESOGASTROPODA

Cerithiidae

Litiopinae

Litiopa melanostoma Rang, 1829, and
Alaba incerta (Orbigny, 1842)
[+ *Rissoa tervaricosa* C. B. Adams, 1845]

(Plates 2 - 4)

These two species are considered together because their veligers and larval shells are so similar that I confused them initially. Originally, I thought I had discovered sculptural and size dimorphism in larval *Litiopa,* but I could not find the dimorphism among protoconchs on postlarval specimens. My identification of larval *Alaba incerta* is made on the basis of optical and SEM comparisons of protoconchs.

Litiopa melanostoma is a circumtropical species whose postlarvae live on floating *Sargassum. Alaba incerta* is known only from the tropical and subtropical western Atlantic (Abbott, 1958: 40–41) and has less specific but benthic substrate requirements; the postlarvae live on the leaves of marine monocotyledons and among algae.

Lebour (1945: 467–468; fig. 8) found larval *Litiopa melanostoma* near Bermuda from June to October, when it was "the commonest veliger in the open water plankton." During April they are also abundant, more so than the *Alaba incerta* veligers occurring with them. I detected no differences between living larvae of the two species, and Lebour's good drawing (fig. 8c) could represent either species. Their similarly shaped larval shells have a sinusigerous outer lip not shown or mentioned by Lebour (Plate 3, Figure *12*). *Sinusigera reticulata* Craven (1877: 111; plt. 3, figs. 3a, b, c), described from the Indian Ocean, undoubtedly is larval *Litiopa melanostoma.* So also is the "Schlanke Sinusigera mit skulpturirter Conchiolinschale" described, discussed and illustrated by Simroth (1895: 94–96; plt. 8, figs. 3–4).

There are two principal larval shell differences between the two species: 1) the full-grown larval shells of *Litiopa melanostoma* (averaging about 0.6 mm long) are larger than those of *Alaba incerta* (averaging less than 0.5 mm long), and 2) on *Litiopa melanostoma* there are regularly spaced, curved crests between the axial ribs, while on *Alaba incerta* there are instead minute pustules aligned parallel with the growth lines (Plate 2). The sculpture of both species is otherwise closely similar. Basal sculpture of both is variable. The growth lines above the sharp keel at the shoulder are orthocline, but below the keel they are opisthocline and at an angle of about 30° to the axial ribs (Plate 2, Figures *7–8*). On *Litiopa* and *Alaba,* there are about 22 axial ribs or subsutural plicae per whorl (Plate 3, Figures *9–11*) ; this number increases to 28 on the last whorl of *Litiopa.* The crests between the axial ribs on *Litiopa* are curved so as all to be concave adapically, and are so aligned as to form spiral series that gradually move abapically across the whorls; each crest edge is directed adapically. Lebour does not mention or show crests between the axial ribs (or "fine longitudinal striae" in her description), but the shell length of the largest of her veligers (0.64 mm) makes it probable that she did have *Litiopa.* The shells and bodies of adult *Litiopa melanostoma* range in color from yellowish buff through amber to chestnut brown; the larval shell colors vary likewise.

Lebour reported that in *Litiopa* "the extreme apex . . . of about a whorl and a half is quite unsculptured" (Simroth had written that it was "strukturlos"). Abbott reported that in *Alaba* the "first two nuclear whorls . . . [are] smooth [and] glossy." The SEM shows at high magnifications that both species have an embryonic whorl with distinctive microsculpture, pits and minute granulations (Plate 4, Figures *13–15*). The pits are irregularly positioned (in places fused), circular or polygonal, and most of them are between 1.0 and 1.5 microns in diameter; the intervening crests vary in width and height. The whole surface is minutely granulated, the granules being in the order of 0.1 to 0.2 micron in diameter and tending to be aligned.

The stage at which the embryo hatched from the egg mass and became pelagic must be recorded by one or the other of two shell features: 1) the area where the pits and minute granules grade into a smooth surface at a shell diameter of 0.07 to 0.09 mm, and where two faint growth lines show on Plate 4, Figure *14,* the first most distinct towards the beginning of the suture; or 2) where subsutural axial plicae suddenly first develop at a shell diameter of 0.10 to 0.11 mm, and where there is a second or third growth line about one third or one half of a whorl beyond the preceding one or two growth lines. Lebour figures (fig. 8b) the "shell of [a] newly hatched veliger 0.08 mm. across."

The conchiolinous, axially multispiral larval opercula of the two species appear identical. Exteriorly, the SEM reveals little (the suture is fairly indistinct) but interiorly the area where the muscle fibers are attached shows clearly (Plate 4, Figure *16*). At higher magnifications, this

area can be seen to contrast with its surroundings by being a porous surface.

Litiopa melanostoma and *Alaba incerta* are the type-species of their respective genera (Wenz, 1940: 753–755; Palmer, 1942). *Alaba* has been placed in the Cerithiopsinae (or Cerithiopsidae) and Diastomatinae (or "Diastomidae") as well as in the Litiopinae. The close similarity in larval shell morphology between *Litiopa* and *Alaba* is evidence favoring the last grouping, a conclusion also reached long ago by A. Adams (1862) from other data.

Janthinidae

Janthina janthina (Linnaeus, 1758), and *Janthina pallida* Thompson, 1840 [*J. rosea* Anton, 1839 (not a *nomen nudum* as stated by Laursen, 1953: 31) may be a prior name for this species]

(Plates 5, 6)

These two species commonly co-occur in the tropical and subtropical western Atlantic, where the postlarvae appear each winter and spring. Like all five species in the

Explanation of Plate 2

Larval *Litiopa melanostoma* Rang (Figures *5* and *7*) [ANSP 320985] and larval *Alaba incerta* (Orbigny) (Figures *6* and *8*) [ANSP 320983].

Figures *5* and *6*: Apertural views (both × 230).
Figures *7* and *8*: Enlargements of the same two shells showing the sculpture between two axial ribs on the last whorl (both × 2 000).

Explanation of Plate 3

Larval *Litiopa melanostoma* (Figures *9* and *10* [ANSP 320985] (both × 230) and larval *Alaba incerta* (Figures *11* and *12*) [former ANSP 313755; latter ANSP 320983] (both × 300).

Figures *9* and *11*: Apical views (latter of protoconch).
Figure *10*: Basal view.
Figure *12*: Oblique apertural view, showing sinusigerous outer lip. Same shell as in Figure *6*.

Explanation of Plate 4

Larval *Litiopa melanostoma*. ANSP 320985.

Figure *13*: Side view of embryonic whorl and of parts of first two postembryonic whorls (× 1000). Same shell as in Figure *5*.
Figure *14*: Apical view of embryonic whorl and of early postembryonic whorls (× 700). Same shell as in Figure *9*.
Figure *15*: Microsculpture of embryonic shell (× 5 000). Same shell and orientation as in Figure *13*.
Figure *16*: Internal view of the conchiolinous operculum of either *Litiopa* or *Alaba*, showing the pale muscle attachment area (×400).

Explanation of Plate 5

Larval *Janthina janthina* (Linnaeus). ANSP 320980.

Figure *17*: Tilted protoconch and part of surrounding teleoconch (× 100).
Figure *18*: Sculpture on part of last whorl in side view (×1 000).
Figure *19*: Apical view of protoconch partly broken out of teleoconch (× 160).
Figure *20*: Apical view of embryonic whorl and part of first postembryonic whorl (× 600).

Explanation of Plate 6

Larval *Janthina pallida* Thompson.

Figure *21*: Tilted protoconch and part of surrounding teleoconch (× 150). ANSP 320981.
Figure *22*: Apertural view of larval shell (× 200). ANSP 320986.
Figure *23*: Apical view of protoconch broken out of teleoconch (× 250). ANSP 320981.
Figure *24*: Sculpture on part of last whorl (× 1 000). Same shell and orientation as in Figure *22*.

Figure 5

Figure 6

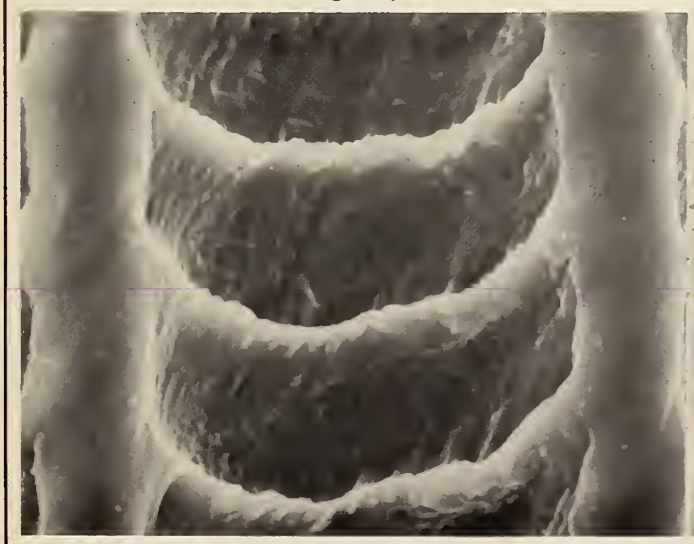

Figure 7

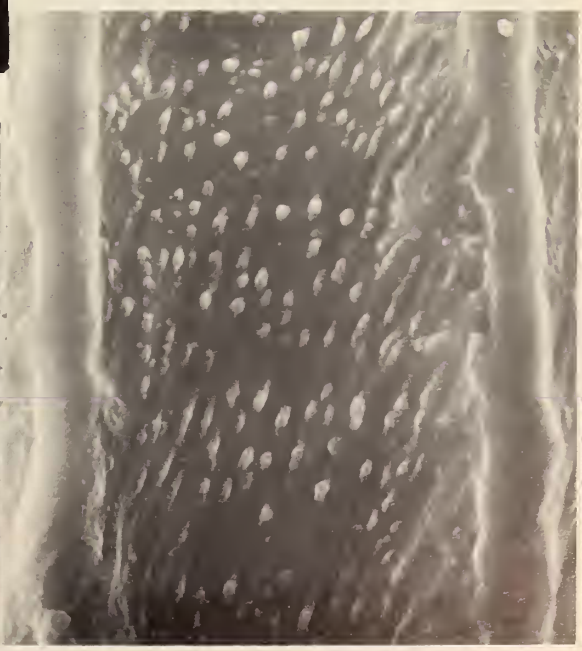

Figure 8

Figure *9*

Figure *10*

Figure *11*

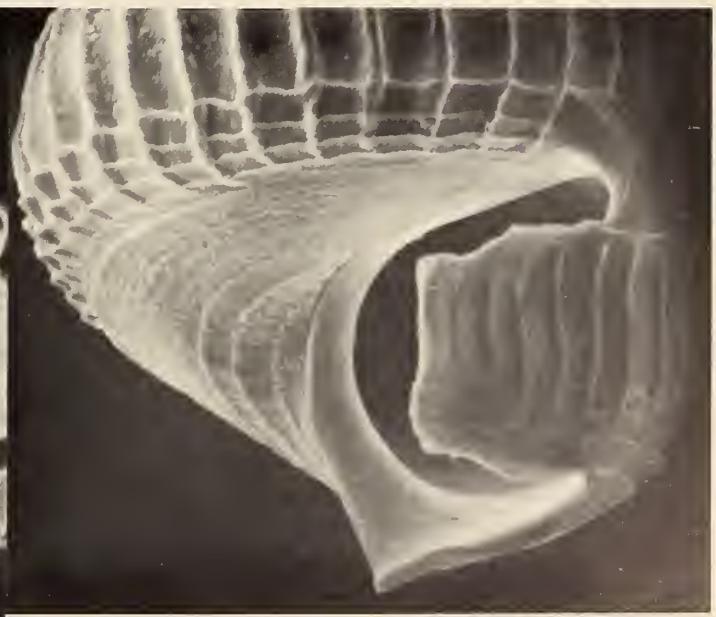

Figure *12*